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COLLEGE OF MEDICINE AND HEALTH SCIENCES

INSTITUTE OF PUBLIC HEALTH

DEPARTMENT OF HUMAN NUTRITION

CHANGE OF HEMOGLOBIN AND ITS DETERMINANTS AMONG VISCERAL LEISHMANIASIS PATIENTS WITH AND WITHOUT IRON AND FOLIC ACID SUPPLEMENTATION NORTHWEST, ETHIOPIA 2017

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Change of hemoglobin and its determinants among visceral leishmaniasis patients with and without iron and folic acid supplementation Northwest, Ethiopia 2017

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List of abbreviation/Acronyms

CI	Confidence Interval
DND*i*	Drug for Neglected Disease initiative
GCP	Good Clinical Practice
HCT	Hematocrit
HGB	Hemoglobin
HIV	Human Immune Deficiency Virus
IDA	Iron Deficiency Anemia
IQR	Interquartile Range
LRTC	Leishmaniasis Research and Treatment Center
MCV	Mean Cell Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
MSF	Médecins sans Frontières
PM	Paramomycine
RBC	Red Blood Cells
RK39	Recombinant Kinesine-39 test.
SD	Standard deviation
SAG	Sodium Antimony Gluconate
SGPT	Serum Glutamate-Pyruvate Transaminase
SGOT	Serum Glutamate Oxalate Transaminase
SSG	Sodium Stibogluconate
SPSS	Stastical Package for Social Science
UOG	University of Gondar
U/L	Unit per Liter
VL	Visceral Leishmaniasis
WBC	White Blood Cell

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Abstract

Introduction: Visceral leishmaniasis (VL) without anemia is very rare, means that almost all VL patients have anemia .It occurs due to many factors like sequestration and destruction of red blood cells (RBC) in enlarged spleen and bone marrow, immune mechanism, and alterations in RBC membrane permeability.

Objective: The objective of this study is to determine change in hemoglobin and its determinant factors among visceral leishmaniasis patients with and without iron–folic acid supplementation, in Northwest Ethiopia, 2017.

Methods: Retrospective cohort study was conducted by reviewing all VL patients chart from Jan /2015 to Dec/2016.Data was entered to EpiData version 3.1 and transferred to Stastical Package for Social Science (SPSS) version 20 for analysis. The data was analyzed using independent sample T test and linear regression model. Ninety five percent (95 %) confidence level was used and p-values less than 0.05 considered as statistically significant.

Result: From a total of 602 visceral leishmaniasis patients, 300(49.8%) were from University of Gondar hospital and 302(50.2%) Abdurafi MSF Holland treatment centers. The mean (SD) change in hemoglobin from baseline to end of VL treatment was 0.99(±1.64) and1.61 (±1.88) g/dl with and without iron-folate supplementations respectively with the P-value of <0.0001at 95% confidence level. In multiple linear regression analysis combination of sodium stibogluconate and paramomycine was positive predictor variable. Whereas age, nasal bleeding ,baseline white blood cell and hemoglobin ,end of treatment spleen size and iron–folic acid supplementation were negatively associated with the outcome variable of change of hemoglobin.

Conclusion and recommendation: The use of Iron-folic acid during the active stage of visceral leishmaniasis does not help for the improvement of hemoglobin, rather it delays. Probably the leishmania parasite might use it for survival and growth. Therefore we recommend for scholars to investigate the appropriate time of iron–folic acid supplementation.

Key words: *Anemia, visceral leishmaniasis, Iron-folic acid supplementation and change of hemoglobin*

1. Introduction

1.1 Statement of the problem

Anemia is a condition characterized by decrease in hemoglobin concentration below the acceptable range. It is convenient to use the hemoglobin thresholds defined by the World Health Organization (WHO) or decrease oxygen-carrying capacity to meet physiological needs of the body (1). Anemia is a major global public health problem especially in developing countries. The World Health Organization estimates that anemia affects nearly two billion people all over the world. This means that one quarter of the global population are affected by anemia. Among this, Iron deficiency anemia (IDA) is responsible for approximately 50% of all types of anemia and 800, 000 deaths per year worldwide(2). Anemia causes physical and mental disability in children and in elderly, as well as working capacity reduction in adults. The above figure indicates that, in many countries, anemia constitutes a serious health problem in the population (3).

Visceral leishmaniasis (VL) is one of the major causes of anemia .It is very serious but treatable neglected protozoal parasitic disease. It is potentially fatal and responsible for the deaths up to 40,000 people per year worldwide(4). The annual global burden shows, approximately 0.2 to 0.4 million VL cases(5). More than 90% of VL cases occur in just six countries: Brazil, Ethiopia, India, Bangladesh, South Sudan and Sudan (6). The annual incidence of VL in Eastern Africa ranges between 29,400 to 56,700 cases accounting for approximately 15 % of the global burden (7). It is the second high disease burden next to the Indian subcontinent. Ethiopia is the second largest number of VL cases in sub-Saharan Africa with an estimated annual burden of 2,000-4,500 new cases per year (9).

The burden of VL disproportionately affects on the poorest segments of the global population. Poverty is associated with poor nutrition and other infectious diseases, poor nutrition increase the risk of infection, and clinically manifested disease (10). In Ethiopia more than 93.8 % and 73% of visceral leishmaniasis patients are daily laborer and illiterate respectively indicating that the monthly income is very low (11).

Anemia is the most common clinical presentation among VL patients that occur due to many factors like sequestration and destruction of red blood cells (RBC) in enlarged spleen and bone marrow, immune mechanism, and alterations in RBC membrane permeability have been implicated. Red cell survival and ferrokinetic studies have suggested that hemolysis is the major cause of anemia in VL. Other conditions like long duration of illness, concomitant disease (inflammation), nutritional status and intestinal helminthes are also major contributing factor for anemia in visceral leishmaniasis patients(12). The clinical presentation of VL varies from region to region reflecting differences in parasite strains, vectors, and hosts(6).

Nutritional deficiency macro nutrient and micro nutrient) like iron, vitamin B12, and folate play great role. The coexistence of these micronutrient deficiencies and iron deficiency may causes anemia (13). The prevalence of anemia among VL patients shows more than 90%, and the degree of anemia is moderate to severe (hemoglobin level~7.5 g/dl), and the status can be recovered with anti-leishmaniasis drug within a certain period of time(14).

Despite the national leishmaniasis treatment guideline recommendation to supplement iron and folic acid for the management of anemia(8), there is no VL treatment centers has been applied the recommendation except Abdurafi Medicine Sans Frontiers (MSF) Holland Kala-azar treatment center. However, there was no clear evidence that shows the effect of iron and folic acid regarding to VL patients but there are experimental studies indicates iron is crucial for survival and growth of leishmania parasite(15, 16). In contrary to this dietary iron has no effect but iron overload suppress the parasite replication and makes it attenuated(17).

Therefore our study aims was to answer the question whether iron and folic acid had better improvement for anemia among Visceral leishmaniasis patients or not?

1.2. Literature Review

1.2.1. Anemia among Visceral Leishmaniasis

Norm chromic normocytic anemia is a frequent and clinically significant feature of VL and in this study the serum iron was measured and found to be normal(18). However, in some studies found erythrocytes in VL patients as microcytic and hypo chromic. This could be due to Iron deficiency anemia(19).Another Prospective study conducted at BPKIHS Hospital, Nepal among 50 cases of visceral leishmaniasis 30 of cases showed microcytic red cell and hyper segmented neutrophils on peripheral blood smear as features of megaloblastic anemia. Among 30 cases of VL with megaloblastic features' 15 of them were treated with Sodium Antimony Gluconate (SAG) only and the other 15 treated with SAG, folic acid and vitamin B12 and the rest 20 cases also treated with SAG. After treatment all 50 cases hemoglobin and total lymphocyte count improved .but in those patients treated with SAG, folic acid and vitamin B12 the reticulocyte count reached peak on the 7^{th} day and normalized at end of 4^{th} week(20).

Another Study conducted in animal model (hamsters) that shows the level of iron from blood was significantly decreased and accumulated in liver. In this study there was no significant difference in mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) level between infected and control hamsters(21).

A systematic review shows that the overall prevalence of anemia is higher than 90 %. The degree of anemia in VL is moderate to severe (hemoglobin level ~7.5 g/dl)(14). A case control study conducted in Sudan shows that the mean hemoglobin of the baseline and end of treatment (at 30day) was 6.5g/dl and 9.5g/dl respectively. But the serum iron and total iron binding capacity (TIBC) were not significantly reduced. (22). Mean prevalence of anemia in two studies using hemoglobin (HGB) levels of 11g/dl as cutoffs were 98.8 %(range (96.5-100)(23, 24).Other three studies using similar cutoffs presented high prevalence of anemia(100%.at <10.5g/dl) and 92%≤10g/dl(25, 26). This shows that the prevalence of anemia is very high in VL patients. Whereas six studies described the prevalence of anemia with the HGB level ≤9g/dl with the mean of 83.2% (range 75-87.9%)(27-31).

A retrospective cohort study was conducted among confirmed VL between January 2000 and December 2005 in Belo Horizonte, Brazil: the mean hemoglobin level was 9g/dl and 86.8% of the patients were anemic(32). In Northern Italian retrospective observational study were conducted from January, 2012 to December 2015 ,among 16patients anemia was observed in all (100%) patients(33).In Southwestern Iran retrospective study was conducted in this period (1999-2014) among 380 VL patients, rate of anemia was 87.3%, 7.5% leucopenia, 39.5% leukocytosis and 64% were thrombocytopenia (34). and another retrospective study conducted in Tehran medical hospital ,Iran a total number of 34 children with confirmed VL through 2004-2011 were included in the study ,Of the total children with VL (97.1%) were anemic, 91.2% had thrombocytopenia and (67.6%) leucopenia (35). In a routine VL treatment program in Pokot, Uganda, Medecins Sans Frontieres diagnosed and treated 4,831 patients with visceral leishmaniasis (VL) between 2000 and 2010; The retrospective analysis of their VL patients data showed that the mean hemoglobin level of VL patient was 7.9g/dl(12). Institutional based cross sectional study conducted in North West Ethiopia which shows that among 403 VL patients, 35.6%, 35.1%,42.3% mildly, moderately and severely anemic respectively , indicating that more than 95% of the patients had anemia in this region (11).

Retrospective study conducted in Amudat hospital, Uganda and kachilba health center in Kenya from 2000-2010; all clinical presentation were not consistently recorded in Amudat, the most frequently reported symptoms in Kacheliba among 2301 patients in addition to fever were weight loss (87.8%), loss of appetite (86.8%), enlargement of spleen (77.1%), nasal bleeding (21.9%), vomiting (16.5%)headache (14.6%), abdominal pain (13.0%), abdominal swelling (11.4%), and diarrhea (8.5%). On physical examination, mean spleen size was 12.3 cm below the costal margin in Amudat and 11.4 cm in Kachelib. Other objective signs such as pallor, edema, and jaundice were reported in respectively= 45.8%, 8%, and 2.3% of cases. More than half of the patients were malnourished on admission, with 24.7% severely malnourished and 27.6%(moderately malnourished(12).(by WHO classification criteria).` Institutional based cross sectional study conducted in Northwest Ethiopia which shows that among 403 VL patients 29.3% mildly,31.7% moderately and 39% severely malnourished

(according to world health organization (WHO) classification criteria)and nearly half of the patients also infected with one or more intestinal parasite ,among this 21% accounted hook warm(11).A retrospective study was conducted at a tertiary care centre serving the kumaon region of Uttarakhand from 2010 to 2014 Splenomegally was the most common sign observed in 17 cases (85%), followed by pallor in 15 cases (75%), pyrexia in 14 cases (70%), weight loss in 12 cases (60%), hepatomegally in 8 cases (40%) and jaundice in 4 cases (20%). Bleeding manifestations were seen in 2 cases (10%)(36).In fact bleeding is risk factor for anemia and finally leads to death among VL cases(37).

1.2.2. Determinant factors

The cause of anemia is multifactorial: duration of illness, and Size of spleen significantly correlate hematological features(38). Reduced plasma iron level in the presence of greatly increased iron stores suggests that the reticulo-endothelial hyperplasia is accompanied by abnormal iron retention by macrophages, typical of anemia of chronic diseases. This may limit the marrow response to hemolysis. In Mediterranean population a very rapid onset of anemia with hemolysis is commonly observed(39). Nutritional status is also factor for anemia as the study shows the prevalence of anemia shows that among 403 VL patients 29.3% mildly,31.7% moderately and 39% severely malnourished and nearly half of the patients also infected with one or more intestinal parasite ,among this 21% accounted hook warm(11).

VL with malaria co-infection more severe symptoms compared to mono-infected patients, however had a similar prognosis, if possibly early diagnosis of malaria as a result of systematic testing(40).Emergence of drug resistance further complicates the treatment of visceral leishmaniasis. It is common among HIV/VL for complete cure of patients will require longer hospitalization. generally the overall improvement will be delayed(41). Iron deficiency anemia (inadequate amount of red blood cells caused by lack of iron) is highly prevalent in less-developed countries but also remains a problem in developed countries. Iron is required for hemoglobin synthesis; if iron stores are low due to reasons ranging from malabsorption , RBC production is unable to keep up with the body’s needs(42).VL patients with severe anemia often require blood transfusion

before starting chemotherapy due to weakness, such interventions to manage anemia may be used for VL patients to improve their quality of life and to increase their tolerance to chemotherapy(14).

1.3 Conceptual framework

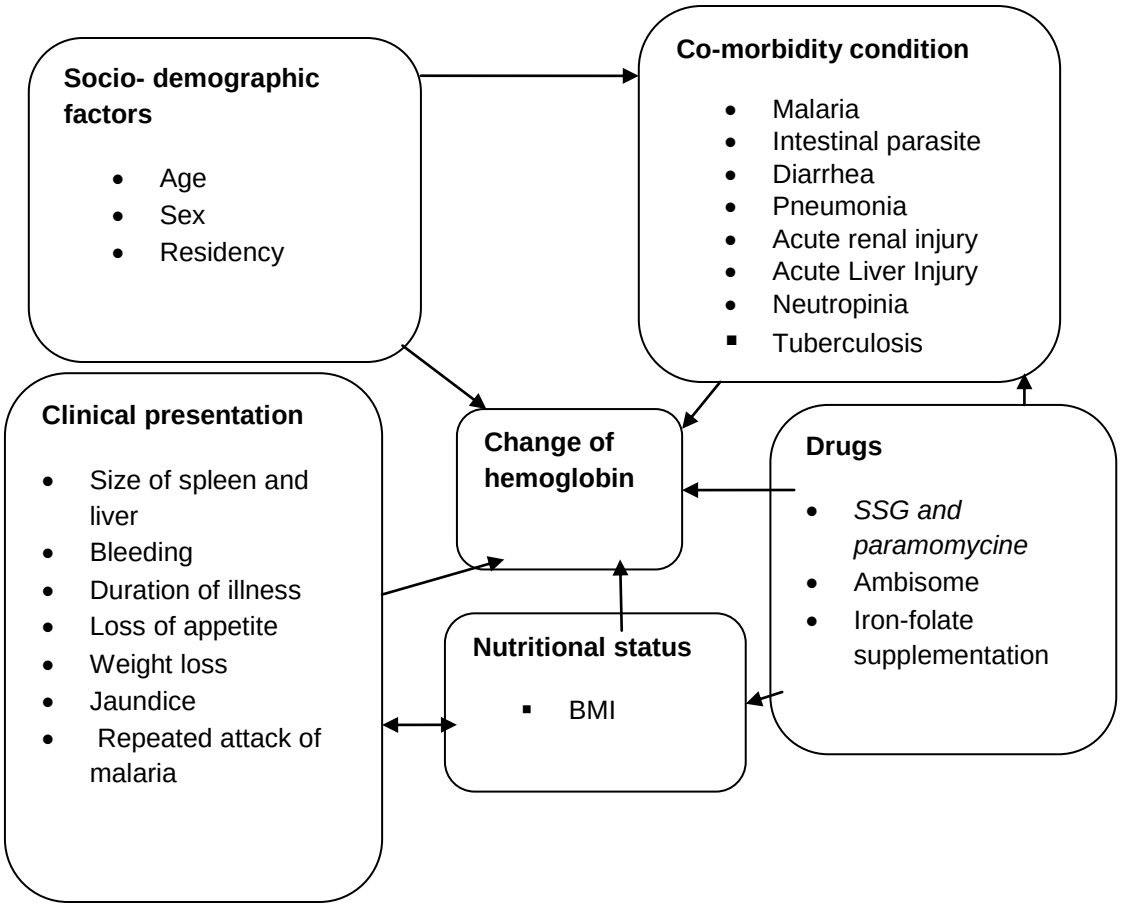

Figure 1 Conceptual framework adopted from literature review

1.4. Justification of the study

Anemia is one of the major clinical presentations of visceral leishmaniasis, and the mean hemoglobin level is 7.2gm/dl(14). It is known that anemia in VL patients can be due to either nutritional deficiency or suppression of erythropeosis due to the sequestration of the parasite in bone marrow and spleen. The management is according to the severity of problem. If the problem is sever, blood transfusion will be given otherwise Iron and folic acid will be supplemented in addition to high iron and vitamin content food.

The national leishmaniasis treatment guideline also recommends iron and vitamin supplementation. However most of visceral leishmaniasis treatment centers did not supplement including University of Gondar Hospital Leishmaniasis Research and Treatment Center except Abdurafi MSF Holland Kala-azar treatment center.

We have observed hematological improvement within two weeks and complete recovery occurs within 4 to 6 weeks in both treatment centers. However, there is no evidence whether iron and folic acid has better treatment outcome of anemia or not among visceral leishmaniasis patients.

Therefore this study will be valuable for local decision maker on the treatment site as well as for researcher to be an input of other studies, specifically to standardize management of anemia among visceral leishmaniasis throughout the country.

2. Objective

2.1. General Objective

- To assess change in hemoglobin and its determinants among Visceral leishmaniasis patients with and without iron–folic acid supplementation in Northwest Ethiopia ,2017

2.2. Specific objective

- To compare changes of hemoglobin among VL patients with and without iron – folic acid supplementation
- To identify determinant factors in change of hemoglobin among VL patients

3. Methods

3.1. Study design and Period

Institution based retrospective cohort study was conducted from March to April/2017 by reviewing VL patient charts from Jan /2015 to Dec/2016.

3.2. Study setting

The Northwest Ethiopia is the high burden VL endemic areas. Addis zemen, Metema, Quara, West Armachiho, Humera, bordering Sudan and Eritrea are the main endemic foci for visceral leishmaniasis. Accounting more than 60% of case burden in the country (43). Sesame is the main cash crop in these regions. Thousands of young migrant workers travel from non endemic area to the endemic foci, Due to the poor economic status and high prevalence of human immune deficiency virus (HIV) in the region, many of them have suppressed immunity and can acquire the disease easily.

The study was conducted in Northwest Ethiopia at the Leishmaniasis Research and Treatment Center (LRTC) of University of Gondar Hospital (UOG) and at the Abdurafi Health Center. Both are the largest as well as specialized leishmaniasis diagnosis and treatment centers in the region and are supported by the non-governmental organizations Drugs for Neglected Diseases initiative (DNDi) and Médecins sans Frontières (MSF) respectively. Each center has its own admition ward, pharmacy, laboratory where hematological and clinical chemistry are done routinely. Patients present to the centers either directly by themselves or are referred from other health institution.

Admitted patients with VL in both treatment centers are routinely evaluated and the findings are documented in their respective chart. The patient's hematology, blood chemistry and other investigations are determined as part of other clinical evaluation modalities. Nutritional assessment, such as weight, and Body mass index are measured weekly and at admission and discharge, respectively.

In Abdurafi MSF Holland kala-azar treatment center in addition to VL management almost all patients are supplemented with Iron and folic acid for anemia management, and therapeutic milk (F100) or Plumpynut for management of malnutrition based on the

severity of the nutritional status in addition to high protein diet. But in LRTC only high protein diet like egg and milk given according to the severity. MSF treatment center has a well established database system for archiving patient information whereas LRTC has a chart archiving room to keep it safely. Hence, data from both treatment centers can be retrieved easily.

3.3. Source population

All patients treated for VL at UOG Hospital and Abdurafi MSF Holland Leishmaniasis Treatment Centers was the source population.

3.4. Study population

The study population was all VL patients treated at UOG Hospital and Abdurafi MSF Holland Leishmaniasis Treatment Centers from 2015 to 2016

3.5. Sample size determination

The sample size was calculated by using mean estimation formula with an assumption of 95% level of confidence, 80% power, a 1:1 exposed: non exposed ratio from pilot study of Abdurafi MSF Holland and UOG Hospital Leishmaniasis Treatment Centers and the mean hemoglobin of non exposed and exposed group were 10.5 and 9.9, ±2.6 respectively.

$$N2 = \frac{\left(Z_{\alpha/2} + z_{\beta}\right)^2 \ \delta^2\left(1 + {}^{1}/_{K}\right)}{\varepsilon^2}$$

N=sample size, δ=Variance, K=ratio of n1 to n2, α= confidence level, β=power

ε=clinically meaningful mean difference of the two groups

N2 $=\frac{(1.96+0.842)^2\ (2.6)^2(1+1/1)}{(10.5-9.9)^2}$ =178, N1=178

The total sample size will be 356 adding 10% for missing data and the final total minimum sample size will be 392. But we have decided to include all the study population who fulfill the inclusion criteria to increase the power.

3.6. Variables of the study

3.6.1. Dependent variables

- Hemoglobin (change of hemoglobin)

3.6.2 Independent variables

Socio-demographic factors, age, sex and residency and hematological and biochemical assessment, clinical presentation of visceral leishmaniasis like fever, weight loss, spleenomegally, loss of appetite, cough, bleeding and bilateral leg edema were considered as independent variable.

Nutritional status, concomitant disease like malaria, tuberculosis, kidney disease, liver disease, intestinal parasite, duration of illness, and iron-folate supplementation were also considered as independent variable.

3.7. Inclusion and exclusion criteria

3.7.1 Inclusion criteria

Visceral leishmaniasis patients age greater than or equal to 18 years and completed anti-leishmaniasis treatment from 2015 to 2016 were included in the study

3.7.2. Exclusion criteria

Visceral leishmaniasis patients with HIV co-infection, patients transfused with blood, pregnant women, and prolonged hospitalization of more than 20 days and discharged before 15 days of VL treatment were excluded.

3.8. Operational definitions

Change of hemoglobin means either increase or decreases a unit of g/dl from the baseline of patient's hemoglobin.

Anemia can be classified by using the level of hemoglobin in g/dl. Hemoglobin status between12–16 g/dl and 13–17 g/dl were considered as normal for female and male patients, respectively. Mild anemia for females and male patients ranged between 11–11.9 g/dl and 11–12.9 g/dl hemoglobin, respectively. For both sexes with hemoglobin level of 8–10.9 g/dl and <8g/dl were considered as moderately anemic and severely anemic, respectively(11).

Body mass index Means weight divided by height in meter square and 18.5 to 24.5; normal, 17 to 18.49; mild, 16 to 17 moderate less than 16; severe malnutrition.

Splenomegally is defined as palpable spleen below the costal margin which was measured along the line of growth in centimeter.

3.9. Data collection procedures

Data extraction tool was prepared and the data collectors were the staffs working in both organizations. They were experienced in data management and handling and trained in Good Clinical Practice (GCP). Four data collectors and 2 supervisors were participated on both treatment sites and training was given for them about the overall purpose of the study and was exercised or demonstrated. During the study period the supervisors as well as principal investigator supervised and correction was given for any unclear things for data collectors.

3.10. Data processing and analysis

The data was entered to EpiData software version 3.1 and it was cleaned, and transformed to Stastical Package for Social Science (SPSS) version 20 for data management and analysis. It was analyzed using independent sample T test for comparison of change of hemoglobin from baseline to end of VL treatment with and without iron- folic acid supplementation. And we used simple linear regression model each possible factors to the outcome variable change of hemoglobin. Variables p-value <0.2, was further analyzed in backward multiple linear regression model after checking the model fitness and fulfilling model assumptions and finally the data presented in the form of tables and texts . Ninety five percent (95 %) Confidence level was used and p-values less than 0.05 considered as statistically significant.

4. Ethical considerations

Ethical approval was obtained from the Institute of public health University of Gondar Research Ethics Committees. Permission was also obtained from LRTC in University of Gondar hospital and MSF Holland in Netherlands to review the records. Data extraction tool was used; patients name was not listed and the confidentiality of patient's data kept and at the end of the study the data collection tool was kept locked.

5. Result

5.1. Socio–demographic and clinical characteristics

A total of 602 VL patients' chart from 2015 - 2016 were reviewed. Almost equal number of cases of VL were included at each study site; three hundred (49.8%) in UOG Hospital and 302(50.2%) in Abdurafi MSF Holland leishmaniasis treatment centers. The vast majority were male (>99.5%) with the mean age of 24.5(±6.2) in both treatment groups. Nearly half of the patients 299 (49.7%) were migrant workers .Whereas the remaining 303(50.3%) were permanent resident in the endemic foci of visceral leishmaniasis. The median (IQR) of duration of illness in both iron and folic acid supplemented (IFS) and unsupplemented (IFUS) groups were 4(2-5) and 4(4-8) weeks respectively.

We have assessed the clinical presentation of both iron and folic acid exposed and unexposed groups of baseline information. This shows that almost all things are equivalent. The main clinical presentation of VL both IFS and IFUS group of patients were summarized in the table below [table 1]. Among IFS fever was 296 (97.7%), loss of appetite, 269(88.8%) weight loss 244(80.5%) and splenomegally 292(96.4%).Similarly the IFUS group also fever was 287(96%), weight loss 244(80.5%), loss of appetite 274(91.6%), splenomegally 286(95.7%). The baseline and end of treatment median (IQR) spleen size IFS group were 7(4-10) and 2(0-5) respectively. Whereas the IFUS group similarly the median (IQR) of spleen size were 8(6-12) and 4(0-6) respectively as well. With regards to nutritional status of VL patients, the overall prevalence of malnutrition in both IFS and IFUS groups were 221(72.9%) and 265(89%) respectively. Of which 52(17.2%) and 122(40.8%) in IFS and IFUS groups were severely malnourished respectively [Table-1].

Table 1 Scio-demographic and baseline clinical characteristics of visceral leishmaniasis patients from 2015-2016 in university of Gondar Hospital and Abdurafi MSF Holland leishmaniasis treatment centers

Variables	Iron and folic acid N=602	
	Supplemented (n=303) Frequency (%)	Unsupplemented(n=299) Frequency (%)
sex		
Male	301(99.3)	298(99.7)
Female	2(0.7)	1(0.3)
Age in years		
18-27	238(78.5)	218(72.9)
28-37	47(15.5)	64(21.4)
38-47	12(4)	16(5.4)
48-57	6(2)	1(0.3)
Mean((±SD*)	24.43(6.15)	24.75(6.0)
Residency		
Stable residents	254(83.8)	49(16.4)
Migrant workers	49(16.2)	250(83.6)
Treatment site		
**LRTC	0	299(100)
*** MSF	303(100)	0
Duration of illness(weeks)		
≤ 6weeks	230(75.9)	192(64.2)
>6weeks	73(24.1)	107(35.8
Median(IQR****)	4(3)	4(4)
Fever >2Weeks		
Yes	296(97.7)	287(96)
No	7(2.3)	12(4)
Abdominal swelling		
Yes	196(64.7)	116(38.8)
No	107(35.3)	183(61.2)
Easy fatigability		
Yes	194(64)	179(59.9)
No	109(36)	120(40.1)
Repeated attack of malaria		
Yes	35(11.6)	119(39.8)
No	268(88.4)	180(60.2)
Weight loss		
Yes	244(80.5)	274(91.6)
No	59(19.5)	25(8.4)
Loss of appetite		
Yes	269(88.8)	270(90.3)
No	34(11.2)	29(9.7)

Nasal bleeding		
Yes	167(55.1)	68(22.7)
No	136(44.9)	231(77.3)
Diarrhea		
Yes	41(13.5)	36(12)
No	262(86.5)	263(88)
Cough		
Yes	157(51.8)	143(47.8)
No	146(48.2)	156(52.2)
Pale conjunctiva		
Yes	230(75.9)	198(66.2)
No	73(24.1)	101(33.8)
Splenomegally		
Yes	292(96.4)	286(95.7)
No	11(3.6)	13(4.3)
Hepatomegally		
Yes	61(20.1)	72(24.1)
No	242(79.9)	227(75.9)
Lyphadnopathy		
Yes	11(3.6)	7(2.3)
No	292(96.4)	292(97.7)
Jaundice		
Yes	20(6.6)	26(8.7)
No	283(93.4)	273(91.3)
***** BLPP edema**		
Yes	38(12.5)	67(22.4)
No	265(87.5)	232(77.6)
******Baseline BMI(kg/m$^{2)}$**		
Normal	82(27.1)	33(11)
Mild malnutrition	96(31.7)	67(22.4)
Moderate malnutrition	73(24.1)	76(25.4)
Severe malnutrition	52(17.2)	122(40.8)

Ee *standard deviation, **Gondar leishmaniasis research and treatment center, ***medicine sans frontiers (Abdurafi), ****inter quartile range, ***** bilateral pedal pitting edema, ******body mass index

We have assessed the diagnostic mechanism of both treatment centers, the majority of patients 283(93.4%) in Abdurafi MSF Holland were diagnosed by serological and using case definition. Whereas in LRTC 269(90%) were diagnosed by parasitological examination. Among this 220(73.6%) was tissue aspiration from spleen and the remaining 49(16.4%was from bone marrow aspiration. The majority of patients IFS and IFUS groups 215(71%) and 269(90%) were treated with combination of sodium stibogluconate and paramomycine injection for 17 days respectively. In addition to VL treatment and IFS, 205(67.7%) were supplemented with therapeutic nutrition

(Plumpynut and f100). At the end of treatment 294(97%) and 295(98.7%) of IFS and IFUS groups were declared initial cure respectively, while the remaining was treatment failures [table-2].

Table 2 Diagnoses, management and concomitant disease or complication of visceral leishmaniasis from 2015-2016 in University of Gondar Hospital and Abdurafi MSF Holland leishmaniasis treatment centers

Characteristics(variables)	**Iron and folic acid**	
	Supplemented Frequency (%)	Unsupplemented Frequency (%)
Diagnosis		
Serological and /or clinical	283(93.4)	30(10)
Parasitological	20(6.6)	269(90)
Site of aspiration		
Spleen	17(5.6)	220(73.6)
Bone marrow	3(1)	49(16.4)
Anti leishmaniasis given		
*SSG+PM	215(71)	269(90)
Ambisome	88(29)	30(10)
Initial treatment outcome		
Initial cured	294(97)	295(98.7)
Failure	9(3)	4(1.3)
Nutrition support(PPN,F100)**		
Yes	205(67.7)	3(1)
No	98(32.3)	296(99)
Concomitant diseases/complications		
Current malaria	15(5)	31(10.4)
Pneumonia	83(27.4)	42(14)
Ear infection	13(4.3)	12(4)
Intestinal parasite	58(19.1)	64(21.4)
Skin fungal infection	3(1)	19(6.4)
Pancreatitis	2(0.7)	10(3.3)
Sever Neutropinia (<500 neutrophils count)	65(21.5)	43(14.4)
***Others	25(8.3)	12(4)

*sodium stibogluconate +Paramomycine, **Plumpynut, therapeutic Milk ***ascities Hyper active spleenomegally, upper respiratory infection, acute kidney injury, oral candidacies, snake bite, hemorrhoid, sexual transmitted infection and tooth infection

5.2. Hematological profile of baseline and end of treatment of VL patients

This research evaluated the baseline and end of treatment hematological profile of patients in both treatment centers. The overall prevalence of anemia was 303(100%) and 300(99%) at the baseline and end of treatment among IFS group respectively. It looks no deference from baseline to end of treatment. But severe anemia at the baseline was 147(48.5%) and 90(29.7%) at the end of treatment. This shows that there was Improvement across the degrees of anemia. Similarly, the overall prevalence of anemia among IFUS groups was 291(97.4%) and 278(93%) at baseline and end of treatment respectively. Here also severe anemia was 107(35.8%) at the baseline and improved to 34(11.4%) at the end of VL treatment. Therefore this showed us improvement of hemoglobin from severe condition to moderate as well mild anemia. We have observed the improvement of other hematological parameters, like the mean (SD) white blood cells from 1.98(± 1.4) to 3.4(±1.5) times 10^3/µl in IFS group and 2.3(±1.3) to 3.8(±1.4) in IFUS group. The median (IQR) platelet count of VL patients improved from 85(60-127) to190 (153-253) times10^3/µl among IFS group. Whereas the IFUS group was 76(46-114) and 228(154-306) times 10^3/µl at baseline to end of treatment respectively [table 4].

Table 3 Comparison of level of anemia from baseline from baseline to end of VL treatment with and without iron and folic acid supplementation

Level of Anemia	**Iron and folic acid supplementation(N=602)**			
	Supplemented(n=303)		Un-supplemented (n=299)	
	Baseline	End of Tx	Baseline	End of Tx
Mild anemia	12(4%)	29(9.6%)	43(14.4%)	96(32.1%)
Moderate anemia	144(47.5%)	181(59.7%)	141(47.2%)	148(49.5%)
Severe anemia	147(48.5%)	90(29.7%)	107(35.8%)	34(11.4%)
Over all prevalence	303(100%)	300(99%)	291(97.4%)	278(93%)

Table 4 Laboratory as well as physical characteristics of VL patients from 2015-2016 in University of Gondar Hospital and Abdurafi MSF Holland leishmaniasis treatment centers between iron and folic acid supplemented and unsupplemented groups

Variables	Iron and folic acid	
	Supplemented group	**unsupplemented group**
WBC(x10^3/µl)		
Baseline mean(SD)	1.98(±1.4)	2.3(±1.3)
End of Tx mean(SD)	3.4(±1.5)	3.8(±1.4)
Platelet count(x10^3/µl)		
Baseline Median(IQR)	85(60-127)	76(46-114)
End of Tx Median(IQR)	190(153-253)	228(154-306)
Mean cell volume (%)		
Baseline Mean(SD)	79.2(±7.3)	82(±9.3)
End of Tx m mean(SD)	81(±9)	85.5(±8.56)
Spleen size(centimeter)		
Baseline Median(IQR)	7(4-10)	8(6-12)
End of Tx Median(IQR)	2(0-5)	4(0-6)
Body mass index(Kg/m^2)		
Baseline Mean(SD)	17.4(±1.6)	16.5(±1.6)
End of Tx mean(SD)	17.9(±1.8)	16.89(±1.64)

WBC=white blood cell, IQR =Interquartile range, SD= standard deviation, MCV =mean cell Volume, SGPT =serum glutamate Pyruvate Transaminase, SGOT=serum glutamate oxalate Transaminase, BUN = blood urea nitrogen, Tx=treatment No anemia, mild, moderate and severe anemia means hemoglobin >13g/dl,11-12.9g/dl, 8-10.9 and <8g/d respectively (WHO classification criteria

5.3 Comparison of change of hemoglobin between two groups

Comparison of change of hemoglobin of VL patients with and without iron-folic acid supplementation is summarized in [table-5]. The base line mean (SD) hemoglobin of 303 (50.3%) of VL patients supplemented with iron folate was 8(±1.7), whereas it was 8.99(±1.67) g/dl at the end of treatment. Similarly, the mean (SD) of hemoglobin of VL patients 299(49.7%) not supplemented with iron-folic acid was 8.77(±2.18) and 10.38(±1.85) g/dl for baseline and end of treatment, respectively. Therefore, the change of mean (SD) hemoglobin with and without iron-folate supplementation from end of treatment to the baseline was 0.99(±1.64) and 1.61(±1.88) g/dl, respectively with the p-value of <0.0001.This means that, there is statistical significant difference between iron-folate supplemented and un-supplemented with the outcome variable of change of hemoglobin [table 5].

Table 5 Comparison of change of hemoglobin with and without iron-folic acid supplementation

Variables		Hemoglobin(g/dl)				95%CI of the mean difference		P-value
Iron- folate supplementation	N	Baseline mean(SD)	End of treatment mean(SD)	change of mean(SD)	Mean difference	Lower	upper	
Yes	303	8.0(±1.7)	8.99(±1.67)	0.99(±1.64)				
No	299	8.77(±2.18)	10.38(±1.85)	1.61(±1.88)	0.62	0.34	0.90	<0.0001

***Independent sample T test done , HGB= hemoglobin, SD =standard deviation , N =sample size

5.4. Determinant factors for the change of hemoglobin

The determinant factors for change of hemoglobin from baseline to end of treatment were assessed using linear regression model. The independent variables with P- value < 0.05 were further analyzed using the backward multiple linear regression model to identify the predictor variables. Combination therapy (SSG+PM) was positively associated with p-value of less than 0.0001. Whereas age (p=0.001), nasal bleeding (P=0.035), baseline white blood cells (p=0.002), baseline hemoglobin (p<0.0001), end of treatment spleen size and iron-folic acid supplementation were negatively associated with the p-value less than 0.0001 at 95% confidence level [table-6].

Based on this analysis result, baseline spleen size, baseline and end of treatment body mass index, did not show statistical significant association with the outcome variable (change of hemoglobin).

Table 6 Determinant factors for change of hemoglobin among visceral leishmaniasis patients

Independent variables (factors)	**(B)Coefficients/std-error**	**95%(CI for B)**		**P-value**
		Lower	Upper	
Constant	6.866/0.397	6.085	7.646	< 0.0001
*SSG+PM(anti-leishmaniasis)	0.710/0.15	0.416	1.005	<0.0001
Age	-0.030/0.009	-0.049	-0.012	0.001
Nasal bleeding	-0.261/0.123	-0.502	-0.019	0.035
Baseline **WBC	-0.139/0.044	-0.226	-0.052	0.002
Baseline ***HGB	-0.513/0.031	-0.574	-0.452	<0.0001
End of *****Tx spleen size	-0.059/0.015	-0.088	-0.029	<0.0001
Iron-folic acid supplementation	-0.574/0.163	-0.895	-0.253	<0.0001
Baseline platelet count	0.001/0.001	-0.001	0.003	0.227
Baseline spleen size	0.024/0.020	-0.016	0.064	0.247
Baseline body mass index	0.006/0.040	-0.072	0.085	0.872

*sodium stibogluconate and paramomycine, ** white blood cells, ***hemoglobin, ****Treatment

6. Discussion

Anemia is the most common public health problem, it has global prevalence of 32.9% and caused 68.36 million years lived with disability. Iron deficiency is commonly assumed to cause half of all cases of anemia(2). However the impact of iron intervention is lower in population with both with high burden of anemia and very high inflammation exposure as well as its absorption is restricted in the presence of inflammation. The consequences of anemia Leads physical and mental disability in children and in the elderly, along with reduction in working capacity in adults(3).The base line and end of treatment mean (SD) hemoglobin of VL patients supplemented with iron folate was of 8(±1.7) and 8.99(±1.67) g/dl, respectively. Similarly the mean (SD) hemoglobin of VL patients 299(49.7%) not supplemented with iron- folic acid was 8.77(±2.18) and 10.38(±1.85) g/dl for baseline and end of treatment respectively. This study illustrated that there was a significant variation in the change of the mean hemoglobin level at the beginning and end of treatment of VL among the patients who took and did not receive iron-folate supplementation (0.99(±1.64) and 1.61(±1.88)g/dl respectively with P-value of <0.0001 at 95% confidence level. The study conducted in Sudan among those who did not receive Iron and folic acid the mean change of hemoglobin was 3.1g/dl which is higher than ours. This might be due to low sample size in Sudan(22). The variation could be attributed by iron supplementation. There is no evidence whether iron directly affects the change of hemoglobin but experimental studies showed that leishmania parasite directly used iron for survival and growth(16). Therefore iron supplementation might create conducive environment for the parasite. Whereas baselines mean hemoglobin difference might be due the hematology analyzer machine as well as the reagent used.

As Backward linear regression model indicates, that those who were treated with combination of sodium stibogluconate and paramomycine, the average change of hemoglobin was increased by 0.710 at 95% CI(0.416,1.005) with the p-value <0.0001 relatively with who treated with Ambisome keeping the other factors constant .which is consistent the study conducted in Sudan that SSG significantly increase the hemoglobin(22).

This could be due to the treatment directly kill the parasite inside phagolysosome by inhibiting trypanothoine reductase (an enzyme that recycle oxidized trypanothoine to keep the trypanothoine in reducing state and the hematopoietic were suppressed with the parasite, As the result of the treatment the hemoglobin start to increased(41). The other possible reason might be those who were treated with Ambisome were relatively low in number.

Age of VL patients is main predictors ,here in this study when age increased in year the average change hemoglobin decreased by 0.03 at 95% CI (-0.049, -0.012) with the p-value of 0.001 keeping other factors constant. It is consistent with the study conducted in East Boston(44).

The size of spleen at the end of VL treatment and average change of hemoglobin were inversely proportional and the coefficient was -0.059 at 95% CI (-0.088, -0.029) with the p-value of <0.0001.This might be due to sequestration and destruction of red blood cells in enlarged spleen and bone marrow(12).

Visceral leishmaniasis patients those had nasal bleeding decreased the average change of hemoglobin by 0.261 at 95%Cl (-0.502, -0.019) with the p-value 0.035 Keeping other factors constant. This could be due to very low platelet count as the result of bone marrow suppression. So that the improvement of hemoglobin might not be the same as to those did not have nasal bleeding.

Iron and folic acid supplementation of VL patients decreased the average change of hemoglobin by 0.574 at 95% CI (-0.895, -0.253) with the p-value <0.0001by keeping the other factors constant. As to my best knowledge there is no study done on Iron-folate among visceral leishmaniasis patients to compare our findings, however there are experimental studies that showed us the effect of iron among leishmania parasite in vivo on animal model as well as in vitro by preparing the culture media. Eventhough there are studies that support our findings, there are also contradictors. The study conducted in Belgium was suggested that adequate supply of iron is needed for the life cycle of leishmania parasite. The authors investigated the effect of iron deprivation on the growth of promastigotes stages of leishmaniasis in vitro using an iron chelating

approach. All chelators tested reduced the rate of promastigotes multiplication in a dose-dependent fashion. Finally they suggested that iron depletion may be an effective mechanism against leishmania infection. And also iron ions are crucial for anti-oxidizing function and other metabolic reaction of the parasite which makes the replication favorable(16).

A study conducted in Portugal the mammalian stage of Leishmania (amastigotes), must acquire iron from molecules accessing the macrophage parasitophorous vacuole (PV) where they inhabit. The molecules include non-heme and heme-bound forms of iron. Here the authors demonstrated that, Leishmania amastigotes are also capable of exploiting iron from hemin and hemoglobin for nutritional purposes. Moreover, evidence is presented that a ligand at the surface of amastigotes binds hemin with high-affinity(15).Leishmaniasis is Intracellular pathogens which employ several strategies for iron acquisition from host macrophages for survival and growth, whereas macrophage resists infection by actively sequestering iron. Here, instead of allowing macrophage to sequester iron, protozoan parasite Leishmania donovani (LD) uses a novel strategy to manipulate iron uptake mechanisms of the host and utilizes the taken up iron for its intracellular growth. Therefore, intracellular LD directly scavenges iron from labile iron pool of macrophages(45).In contrary another study conducted in Portugal, the effects of iron supplementation or deprivation on the growth of L.infantum. The authors confirm that dietary iron deficiency did not affect the protozoan growth, whereas iron overload decreased its replication in the liver and spleen of a susceptible mouse strain(17).

7. Limitation of the study

This research used secondary data, as the result, did not assess the income as well as dietary history of patients and which it might reduce the validity. The other limitation was unable to show the effect of iron and folic acid on the change of hemoglobin separately.

8. Conclusion

Anti leishmaniasis treatment of SSG and pm, age, nasal bleeding, baseline white blood cells, baseline hemoglobin and iron and folic acid supplementation were determinants of change of hemoglobin.

Iron-folate supplementation has no significant effect on the improvement of VL patient's hemoglobin level, rather it delays. However, it does not mean that there is no iron- folate deficiency. Probably the leishmania parasite might use iron for survival and growth.

9. Recommendation

For clinician: Combination of SSG and PM for the management of VL patients had direct relationship with the change of hemoglobin which means that treatment of VL alone boost up the hemoglobin level. Whereas age of the patients, baseline WBC, baseline hemoglobin, end of treatment spleen size, nasal bleeding and Iron and folic acid supplementation negatively affects the improvement of hemoglobin. Therefore we recommend for clinicians to focus and prompt management on the above determinants for the better improvement of anemia.

For researcher: Here in this study we couldn't show the effect of iron and folic acid separately. Therefore we recommend scholars to investigate and explore more on the effect of each as well as the appropriate time of supplementation among VL patients with randomized control study design.

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Annex I: Case report format for data collection

Visceral Leishmaniasis data collection tool for Change of hemoglobin among VL patients with and without iron-folate supplementation for those who were admitted and treated for VL and fulfill the inclusion criteria from Jan/2015 to Dec /2016.

Investigator:-Tadele Mulaw

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1. Scio-demographic characteristic of the patient

101	Patient ID	_____	Date _____(dd/mm/yyyy)
102	Age	_____	
103	Sex	1. Male	2. Female
104	Residency	1. Migrant worker	2. permanent resident
105	Treatment site	1. LRTC	2. MSF

2. Baseline history and physical examination

code	Clinical history	Yes	No	Code	Physical examination	Yes	No
201	Duration of illness(in weeks)_____	NA	NA	212	Pale conjunctiva		
202	Fever more than 2 wks			213	Splenomegally		
203	Abdominal swelling			214	Hepatomegally		
204	Easy Fatigability			215	Lyphadnopathy		
205	Repeated attack of malaria			216	Jaundice (icteric sclera)		
206	Weight loss			217	Oedema(bilateral pitting pedal)		
207	Loss of appetite						
208	Nasal Bleeding						
209	Diarrhea						
210	Cough						
211	Yellow discoloration of eyes						

3. Clinical evaluation (Hematological and Biochemical laboratory) findings

Code	Category	Base line	End of treatment	Code	Category	Baseline	End of treatment
301	WBC ($x10^{3/\mu l}$			310	Weight(Kg)		
302	Hemoglobin (g/dl)			311	Height (cm)		NA
303	Platelet($x10^{3}{}_{/\mu l}$)			312	BMI(kg/m^2)		
304	MCV (%)			313	Spleen size(cm)		
305	Neutrophile count(N_o)						
306	SGPT(U/L)						
307	SGOT(U/L)						
308	Creatinine (mg/dl)						
309	BUN(Mg/dl)						

4. Diagnosis and management of VL

Code	Methods of diagnosis	1. Serology and/ or clinical	2. Parasitological
401	If parasitological, where is site?	1. SA 2. BM	3. Lyphnode
402	Anti-leishmaniasis given	1. SSG+Pm	2. Ambisome
403	Initial treatment outcome	1.Initial cured	2. failure
404	Iron-folate supplementation	1. Yes	2. No
405	Plumpynut given	1. Yes	2. No

5. **Concomitant disease**

Concomitant disease		Yes	No
501	Malaria		
502	If "yes "what type of malaria?	1. Pf 2. Pv	3. Mixed
503	Pneumonia		
504	Ear infection		
505	Intestinal parasites		
506	Skin fungal infections		
507	Pancreatitis(physician Judgment)		
508	Viral hepatitis (screened viral marker)		
509	Neutropinia (neutrophile count<500)		
510	Tuberculosis(any)		
511	Others (specify)		

NB: inclusion criteria 09

- *Age ≥18 years and completed treatmen*t

Exclusion

- *HIV/VL*
- *Pregnant mother*
- *Stayed less than 15 days or greater than 20 days*
- *Blood transfused*

Data collector signature _____ supervisor signature _____

Annex II: Declaration

I, the undersigned, senior MSC in applied human Nutrition student declare that this thesis is my original work in partial fulfillment of the requirement for the degree of master of science applied human nutrition.

Name; Tadele Mulaw

Signature_____

Date _____

Place of submission: Institute of Public Health, College of Medicine and Health Sciences, University of Gondar.

Date of submission _____

This thesis work has been submitted for examination with our approval as University advisor(s).

Advisor(s)	Name	Signature	Date
1.	Mr Amare Tariku (MSC, Asst.Prof.)	_____	_____
2.	Mr Adino Tesfahun(MPH)	_____	_____